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Pascale Cossart[1,2,3*] and Alice Lebreton[1,2,3*†]

1. Institut Pasteur, Unité des Interactions Bactéries-Cellules, Paris, France.
2. Inserm, U604, Paris, France.
3. INRA, USC2020, Paris, France.

* Address: Unité des Interactions Bactéries-Cellules, Institut Pasteur, 25 rue du Dr. Roux, 75015 Paris, France. Fax: + 33 1 45 68 87 06.

E-mail addresses: pascale.cossart@pasteur.fr (P. Cossart) or alice.lebreton@ens.fr (A. Lebreton).

† Present address: École Normale Supérieure, Institut de Biologie de l'ENS (IBENS), Inserm U1024 and CNRS UMR 8197, Paris, F-75005 France.

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Abstract

Listeria monocytogenes is a food-borne pathogen causing an opportunistic disease called listeriosis. This bacterium invades and replicates in most cell types, due to its multiple strategies to exploit host molecular mechanisms. Research aiming at unravelling *Listeria* invasion and intracellular lifestyle has led to a number of key discoveries in infection biology and cell biology. In this review, we report on our most recent advances in understanding the intimate crosstalk between the bacterium and its host, resulting from in-depth studies performed over the past five years. We specifically highlight new concepts in RNA-based regulation in bacteria and discuss important findings in cell biology, including a new role for clathrin and an atypical mitochondrial fragmentation mechanism. We also illustrate the notion that bacterial infection regulates host gene expression at the chromatin level, contributing to an emerging field called patho-epigenetics. This review corresponds to the lecture given by one of us (P.C.) on the occasion of the 2014 FEBS | EMBO Woman in Science Award.

Introduction

The causative agent of listeriosis, *Listeria monocytogenes*, has led to a number of discoveries in various biological fields. It was identified by E.G.D. Murray in 1924, as a gram-positive bacillus responsible for epidemic cases of mononucleosis in laboratory animals, and named *Bacterium monocytogenes* [1]. It was later on shown to cause sporadic cases of meningitis in humans [2], before being recognized as an opportunistic, food-borne pathogen of human, cattle and wild animals [reviewed in 3]. *L. monocytogenes* can live freely in the environment, but infects also a wide range of animal hosts including arthropods as well as cold and warm-blooded vertebrates. In mammalian hosts, this bacterium can cross the intestinal, foeto-placental and blood brain barriers, allowing its dissemination throughout the organism (Fig. 1A). Even though listeriosis is not a major health concern for immunocompetent individuals, it can constitute a life-threatening disease in the elderly and in immunocompromised patients; it also has serious outcomes in pregnant women, causing still-birth or frequently lethal neonatal infections [reviewed in 4].

L. monocytogenes has been broadly used by immunologists for its ability to induce a cell-mediated immune response, while antibodies play no role in recovery from infection [5,6]. It has also become a tool for anti-tumour immunotherapies, and is in pre-clinical trials as a live-attenuated vaccine for cancer treatment [7]. *Listeria* atypical immunological properties derive in part from its ability to replicate intracellularly [reviewed in 8]. Indeed, this facultative intracellular bacterium can enter and multiply in the cytosol of most human cell types and spread to neighbouring cells, using an arsenal of virulence factors that target diverse cellular components and subsequently hijack various host cell functions (Fig. 1B). For the past 30 years, unravelling the intracellular lifestyle of *L. monocytogenes* has contributed to a significant number of advances, not only in microbiology and cellular microbiology, by identifying virulence factors and characterizing their functions, but also in cell biology, by elucidating cellular pathways that had remained elusive and giving rise to the discovery of novel molecular mechanisms [reviewed in 9].

The major bacterial effectors responsible for bacterial entry into host cells, escape from the phagocytic vacuole, intracellular motility, as well as their cellular partners, are now well characterized [reviewed in 9,10]. Nonetheless, the past five years of research have brought additional knowledge about the multiple strategies used by *Listeria* to subvert its host functions and programmes. We review here our most recent achievements in the understanding of the host-bacterial crosstalk; we are not aiming at being exhaustive, but instead wish to place significant advances into a broader perspective, including that of work by others. We report how high-resolution transcriptomics of the bacterium allowed the identification of unconventional mechanisms of bacterial gene expression; we discuss how bacterial-induced phenotypes enabled us to reconsider our appreciation of a number of central cellular functions; last, we highlight progress in deciphering the host response to infection and its manipulation by *Listeria*, in particular at the onset of innate immune defences at the chromatin level. As ever, the use of *L. monocytogenes* as a model proves an outstanding tool to generate and establish novel concepts in infection biology, in microbiology and in cell biology.

Discovery of unconventional mechanisms regulating bacterial gene expression.

The bacterial transcriptional landscape examined at high resolution.

Upon infection, the onset of the host-bacterium dialogue translates into a drastic remodelling of both prokaryotic and eukaryotic gene expression programmes. Initial transcriptome studies had focused on *Listeria* grown *in vitro* [11,12]. Later on, high-resolution transcriptomics of bacteria grown in *in vitro*, *ex vivo* and *in vivo*, using tiling microarrays, have provided us with a detailed

insight into the reshaping of the bacterial transcriptional landscape upon switch from saprophytism to virulence [13]. This study has allowed a comprehensive re-annotation of the genome of the *L. monocytogenes* strain EGD-e [14] and the establishment of the first genome-wide operon map. Novel transcriptional units have been defined, in particular antisense RNAs (asRNA), some of which spanning several open reading frames (ORF). 29 novel non-coding RNAs (ncRNA) of less than 500 nucleotides have also been uncovered, adding up to the 21 previously known small RNAs (sRNA). Remarkably, cross-species comparison has revealed that several sRNAs are absent in the non-pathogenic species *Listeria innocua* and display similar expression features as protein-coding virulence genes. Moreover, deletion of some of these sRNA genes impaired virulence in a mouse listeriosis model [13,15], suggesting that sRNAs can participate in the control of virulence. Last, analysis of the transcriptomes of deletion mutants of two major regulators of *Listeria* virulence genes, PrfA and σ^B [16], has highlighted that σ^B plays a key role in switching on the expression of virulence genes in the intestinal lumen, while PrfA triggers the expression of effectors required for intracellular lifestyle when the bacteria are grown in blood [13].

This pioneering work in *Listeria* transcriptomics using tiling arrays was followed by a number of others, which, by analyzing a variety of growth conditions and techniques, contributed to drawing an accurate picture of the bacterial transcriptional landscape and its fine-tuning at different stages of the infection process. For instance, the transcriptional profiling of *L. monocytogenes* recovered from the spleen of infected mice provided important information regarding the metabolic status of the bacterium *in vivo*, the regulatory networks at play in virulence, and allowed the identification of novel candidate virulence genes [17].

An additional degree of resolution in *Listeria* transcriptomes was reached with the use of RNA-Seq, which allowed the mapping of transcription start sites in both *L. monocytogenes* and *L. innocua* [18]. This analysis, performed on bacteria grown in several different growth conditions, has enabled the annotation of additional sRNAs and asRNAs, in addition to all 5'-UTRs. Public access and navigation through these results are facilitated by the *Listeria* browser (http://www.weizmann.ac.il/molgen/Sorek/listeria_browser/). Thanks to an increasing number of high-resolution transcriptomic studies [13,15,18-20], referenced *Listeria* ncRNAs now include 154 trans-acting sRNAs, 46 cis-regulatory RNAs and 104 asRNAs [21]. These ncRNAs constitute a pool of putative regulatory mechanisms for bacterial gene expression, most of which remain to be characterized. Detailed study of some of these transcripts has revealed an amazing versatility in their regulatory properties, which we will illustrate below.

Novel riboswitch-mediated regulations.

Among the new ncRNA elements discovered in the above-described studies, some had been previously annotated in databases as putative riboswitches. Riboswitches are *cis*-regulatory RNA elements, which act most often as sensors of metabolites, but can also bind a variety of specific ligands including metal ions or cyclic-di-GMP. Upon ligand binding, a conformational change in the structure of the riboswitch leads to premature transcription termination, translation arrest, or both. Interestingly, the *L. monocytogenes* LysRS riboswitch has been shown to act not only as a lysine-dependent regulator of the transcription of the downstream gene, but also as a transcription terminator for the upstream gene [13].

Another novel type of riboswitch is the previously named sRNA Rli39 (Fig. 2A). Instead of being classically located in a 5'-untranslated region (UTR), this vitamin B_{12}-dependent riboswitch is located at the 3'-end of a gene, and in antisense orientation to the downstream gene *pocR*. In *Salmonella*, PocR is a transcriptional regulator for genes of the propanediol utilisation pathway – a process requiring B_{12} as a cofactor. PocR also positively regulates its own expression in the presence of propanediol. In-depth analysis of this locus in *Listeria* has revealed that the B_{12}-

dependent riboswitch controls the expression of an asRNA to *pocR*, *aspocR* [22]. In absence of B_{12}, the riboswitch adopts an anti-terminator structure, which allows transcription of *aspocR* and thus blocks that of *pocR*. Conversely, the transcription of *aspocR* terminates in presence of B_{12}. Altogether, this elegant system allows the transcription of *pocR* – and thereby that of PocR-regulated genes – only when both propanediol, the substrate of the pathway, and vitamin $B_{12,}$ the cofactor of propanediol catabolic enzymes, are present in the growth medium.

While riboswitches usually directly regulate their targets in *cis*, the role of the short transcripts terminated upon ligand binding to the riboswitch had remained unexplored; their study has revealed that one such transcript acts in *trans* in the regulation of *prfA* [23]. Transcription termination by a S-adenosylmethionine (SAM) -dependent riboswitch can generate a small RNA, SreA, as a function of the concentration in methionine (Fig. 2B). Independently of SAM binding, base-pairing of SreA to the 5'-UTR of the *prfA* transcript blocks its translation. Conversely, PrfA controls the transcription of SreA, thus creating a negative feedback loop on *prfA* expression. This *trans*-regulation of *prfA* expression by SreA adds to the previously described *cis*-acting thermosensor [24], which unmasks the ribosome binding site (RBS) at 37°C.

Based on these two studies and on bioinformatics predictions, we anticipate that many other ncRNAs may be regulated by riboswitch-dependent mechanisms; the extent of this new level of control in bacterial regulatory networks remains to be assessed.

The "excludon" concept.

As early as the release of the first high-resolution transcriptome of *L. monocytogenes*, a class of very long asRNAs (lasRNA) had been highlighted, which displayed a dual function as both an asRNA and an mRNA [13]. The participation of these long asRNAs (lasRNA) in a novel type of regulation has been confirmed and extended, giving rise to the concept of the excludon (Fig. 2C) [18,25]. Excludon loci harbour a peculiar topology, where two transcriptional units encoding proteins with mutually exclusive functions display a divergent orientation. In this context, the very long 5'-UTR of one gene can span over the divergent transcriptional unit (sometimes, covering several ORFs of an operon) and inhibit its expression, while its coding part is potentially translated. It is highly possible that the excludon concept holds true not only in bacteria, but also in other organisms.

A PNPase-dependent CRISPR system in Listeria.

Even though RNA-mediated regulation of gene expression is an expanding field, not all small RNAs have a function as transcriptional regulators. For instance, clustered regularly interspaced short palindromic repeats (CRISPR) have been described to constitute a prokaryotic adaptive immunity against exogenous nucleic acids. An additional role of these sRNAs in the virulence of pathogenic bacteria is emerging [reviewed in 26,27]. CRISPR processing and function depend on the nuclease or helicase activities of proteins encoded by CRISPR-associated (*cas*) genes. In addition to classical type I and II CRISPR-*cas* systems, all *L. monocytogenes* strains harbour a RliB-CRISPR, which has previously been shown to play a role in virulence [13]. Unlike other CRISPRs, RliB adopts a non-canonical secondary structure and is devoid of *cas* genes [28]; however, part of the processing of RliB-CRISPR to its mature form depends on *Listeria* polynucleotide phosphorylase (PNPase). RliB is functional for defence foreign DNA, in a PnpA-dependent manner, provided *cas* genes from a canonical type I CRISPR-*cas* system are present elsewhere in the genome. The PnpA-dependent regulation of the activity of RliB-CRISPR points to a more versatile mode of action of CRISPR than classically illustrated.

Cell biology processes explored in light of *Listeria* infection.

Most initial studies of *L. monocytogenes* infectious process have focused on host cytoskeleton rearrangements occurring during entry, intra- and inter-cellular movements [29,30]. During entry, which occurs by a zipper mechanism, activation of the cell surface receptors E-cadherin and Met by bacterial proteins, InlA and InlB respectively, triggers cell-signalling cascades, which lead to the recruitment of components of the host cell cytoskeleton at the bacterial entry site [reviewed in 31,32] (Fig. 3A,B). This promotes engulfment of the bacterium into membrane extensions, closing of the phagocytic cup and formation of an internalisation vacuole. After escape from the vacuole, *Listeria* is able to navigate through the host cytosol, *via* the recruitment of the actin-nucleating complex Arp2/3 by the surface-exposed bacterial protein ActA [reviewed in 33]. By doing so, ActA stimulates the polymerisation of a branched actin filament network at the rear end of the bacterium. These filaments generate so-called actin comet tails, which propel the bacterium across the cytoplasm and eventually as protrusions into neighbouring cells.

New advances have been recently made in understanding how the host cytoskeleton is subverted by *Listeria*. As in past studies, analysis of these nano-filaments not only provides insights into the intracellular bacterial lifestyle, but also improves our understanding of the mechanistic role of several cytoskeletal components.

Clathrin function reconsidered.

It has long been thought that clathrin was only involved in the endocytic uptake of small cargo, such as nutrients and signalling molecules [34]. The heavy and light clathrin chains can assemble into triskelions and form a polyhedral lattice around budding membrane vesicles [35]. This dogma was reconsidered when it appeared that clathrin was also involved in the entry of *L. monocytogenes*, other bacteria and large viruses using a zipper mechanism [36-38]. The clathrin-mediated endocytosis machinery can therefore also participate in the internalization of large particles, where it acts as an actin network organizer [reviewed in 39]. Clathrin is involved not only in endocytic pathways, but also in pedestal formation by enteropathogenic *E. coli* (EPEC), a hallmark of *E. coli* colonisation of the intestine [40].

The hierarchical recruitment of clathrin adaptor proteins and actin their was then thoroughly explored, during the entry of *L. monocytogenes* into host cells, or during pedestal formation [41]. In both cases, the adaptor Dab2 is required for the formation of clathrin-coated pits, and the light chain of clathrin recruits the actin-binding factor Hip1R. Bacterial adhesion induces the tyrosine phosphorylation of clathrin heavy chain, a key event in the internalisation process. In parallel, the recruitment of myosin VI by Dab2, and its progression along actin filaments, provides a pulling force for *Listeria* internalization (Fig. 3A).

The function of clathrin as a hub promoting actin cytoskeletal rearrangements has been further extended to other events in cell biology; indeed, the same machinery is involved in the formation of adherens junctions between epithelial cells [42]. Altogether, these findings have led to a paradigm shift: clathrin should no longer be considered as solely involved in endocytosis.

Control of Listeria *entry and actin dynamics by a lipid phosphatase.*

During endocytosis of bacterial pathogens, phosphoinositides play a crucial role in local signalling events at membranes by recruiting cellular effectors involved in entry and activating signalling cascades that control actin dynamics [reviewed in 43]. During InlB-dependent invasion, phosphorylation of phosphatidyl-inositol bisphosphate ($PI_{4,5}P_2$) to trisphosphate ($PI_{3,4,5}P_3$) by the phosphatidylinositol 3-kinase (PI3K) p85/p110 promotes *Listeria* internalisation, through the activation of Rac1 and WASP family members, and stimulation of actin polymerisation [44-46]

(Fig. 3A). A novel player in this pathway, the phosphatidylinositol phosphatase OCRL, has recently been shown to have an opposite effect, consistent with its biochemical activity, which reversed PI3K action. When recruited at the site of bacterial adhesion (Fig. 3B), OCRL restricts bacterial entry by dephosphorylating $PI_{3,4,5}P_3$, thereby inducing actin depolymerisation downstream of Met [47].

Septins: New partners of actin filaments and link with autophagy.

Among other components of the cytoskeleton, septins, a family of GTP-binding proteins which can assemble into heteropolymeric filaments, had been found associated with the *Listeria* phagosome [48]. SEPT9, SEPT2 and SEPT11 localize at the site of *Listeria* as well as *Shigella* entry [49] (Fig. 3A). Surprisingly, SEPT2 is required for bacterial invasion, whereas SEPT11 restricts it, suggesting that different septins may play opposite roles in the tuning of actin-based molecular events [49,50]. Mechanistically, septins influence the availability of Met at the cell surface and modulate the interaction of InlB with its receptor Met, probably by anchoring Met to the actin cytoskeleton [51].

More generally, septin recruitment seems to be a general pattern wherever actin polymerisation is taking place [52]. For instance, septin rings assemble around *Listeria* and *Shigella* actin comet tails [53]. Quite strikingly, septins can also entrap *Shigella* into highly stable cage-like structures. This process is dependent on N-WASP recruitment by bacterial surface protein IcsA and actin polymerisation. Encaged *Shigella* are not motile, and are therefore also restricted in their ability to spread from cell to cell. Septin cage formation and autophagy appear as inter-dependent processes [53,54]. Caging has not been observed for *Listeria*; interestingly, *Listeria* has been shown to escape autophagy thanks to the activity of its surface protein ActA [55], which has led to the hypothesis that ActA allows the bacterium to evade both septin caging and autophagy. One should note that *Listeria* has also evolved a distinct, redundant mechanism to escape autophagy, *via* the surface-exposed protein InlK. This member of the internalin family disguises intracytosolic bacteria from autophagic recognition by interacting with the host major vault protein (MVP) [56].

Together with a number of studies in other systems, the above-reported studies contributed to highlight septins as scaffolds for protein recruitment in numerous biological processes, including host-microorganism interactions. Septins also constitute diffusion barriers for subcellular compartmentalization. Due to their central functions, septins are increasingly considered as the fourth component of the cytoskeleton [reviewed in 57].

Three-dimensional architecture of actin comet tails.

The two-dimensional structure of actin comet tails has been described very early on, both by optical and electron microscopy [30,58], and thorough insight into comet tail composition has been provided by proteomics [59]; however, the three-dimensional organization of these structures remained elusive. The use of cryo-electron tomography has recently revealed the 3D-structure of these tails at the resolution of individual filaments [60] (Fig. 3C). In comet tails as well as in other cellular actin-based assemblies (stress fibres and filopodia), actin is bundled into parallel, hexagonally-packed filaments interspaced by regular 12-13 nm spacing. Together with a report proposing that tangential actin filaments act as primers for explosive actin-network growth [61], this work has contributed to an explanatory model for the initiation of comet-tail assembly and ensuing actin-based motility.

Mitochondrial dynamics: A novel fission mechanism.

In addition to exploring the structure and dynamics of the cell cytoskeleton, research on *L.*

monocytogenes has contributed to deepening our knowledge of several other central cellular processes. For instance, it has led to the identification of an atypical mechanism of fragmentation of the mitochondrial network, which is induced upon *Listeria* infection [62] (Fig. 3D,E). This fragmentation, which is triggered upon calcium influxes due to membrane pores created by the major virulence factor of *L. monocytogenes*, listeriolysin O (LLO), can also be obtained with other pore-forming toxins of the same family. *Listeria*-induced mitochondrial fragmentation is transient and impairs the energy-producing function of the organelle. Functional mitochondrial dynamics is required for efficient infection; indeed decreased infection is observed in cells with impaired mitochondrial fusion, while cells with impaired mitochondrial fission display improved infection. Interestingly, LLO-dependent fission of mitochondria is independent of the dynamin-like protein Drp1. However, similarly to the canonical Drp1-dependent fission, it requires transient contacts with the endoplasmic reticulum, as well as actin polymerisation [63]. The involvement of other cellular components in this atypical fission mechanism is currently being addressed.

Post-translational modifications of host proteins: A role for SUMO in the infection.

The above mentioned toxin, listeriolysin O, which was originally described for its role in the disruption of the phagocytic vacuole after *Listeria* entry, turns out to be a highly versatile virulence factor with multiple effects on host cell functions [reviewed in 64]. One of them is the proteasome-independent degradation of various cellular proteins, such as Ubc9, telomerase and Mitochondrial fission factor (Mff) [63,65,66] (Fig. 3D). Ubc9 is the unique E2-ligase responsible for SUMO conjugation pathways; the modification of proteins by this small ubiquitin-like polypeptide controls their function in key cellular pathways such as transcription, intracellular transport, or stress responses. Ubc9 degradation results in the global de-SUMOylation of cellular proteins [65]. Given that hyper-SUMOylation impairs infection efficiency, we have proposed that some SUMOylated proteins participate in controlling infection, and that LLO-dependent de-SUMOylation prevents their function. Whether the effect on infection depends on the modulation of one or several protein functions or localisations when they are SUMOylated, or on a role of the conjugated SUMO group *per se*, is the object of current investigations.

Novel insights into the host response to *Listeria* infection.

Host response to infection revealed by transcriptomics.

As described above, the transcriptome of *L. monocytogenes* has been investigated in depth at various phases of the bacterial life cycle. In contrast, studies dedicated to the transcriptional response of the infected host remain fragmentary. Nevertheless, several transcriptome analyses of *Listeria*-infected immune [67,68] or non-immune [69,70] cell lines have been reported. Typically, the host transcriptional response corresponds mostly to innate immune signalling pathways, driven by NF-κB and interferons (IFN), with cell-type-specific variations around this core. Insights into more complex signalling events occurring in host infected tissues have been provided by transcriptome studies performed in the intestine of gnotobiotic humanized mice [71]. Another interesting approach allowing the analysis of a homogenous cell population rather than a tissular response has been adopted by Best *et al.*: The authors explored the differentiation process of memory T-cells, by analysing the transcriptome of $CD8^+$ T cells sorted from infected mice [72].

To address the role of commensal and probiotic bacteria in listeriosis, a transcriptome of the host and of *Listeria* has been undertaken, in the germ free "humanized" mouse model of listeriosis after colonisation of the intestine with lactobacilli [73]. It has highlighted that lactobacilli have a protective role against the *in vivo* dissemination of *Listeria*. They influence the transcriptome of the

bacterium, essentially by modulating expression of genes involved in the utilisation of intestinal available nitrogen and carbon sources such as propanediol and ethanolamine. On the host side, lactobacilli not only affect the host immune defences by dampening IFN response; they also strikingly modulate the expression of several microRNAs.

The influence of the microbiota itself has been further investigated using deep-sequencing approaches on RNA extracted from intestinal tissue of infected conventional or germ free mice [74]. This has shown the role of the gut flora in modulating the host microRNA response to infection, and provided a regulatory network defined by host microRNAs and their target genes.

Set-up of host innate immune responses.

An important achievement of the past five years in the understanding of host innate immune responses to infection has been the unravelling by several groups of a number of bacterial pathogen-associated molecular patterns (PAMPs), cellular sensors and signalling pathways resulting in the onset of interferon responses [reviewed in 75]. Among these, secretion of cyclic-di-AMP by *Listeria* appears as a major agonist of the pathway, and signalling occurs via DDX41, STING, TBK1 and IRF3 [76,77]. Bacterial nucleic acids can also be sensed via RIG-I, and then induce STING-dependent IFN responses [78,79]. In murine macrophages, we have uncovered a non-conventional intracellular pathway resulting in the induction of IFNβ, which depends on the toll-like receptor TLR2, the adaptor TRIF, IRF3 and IRF7 [80].

To date, whether the type I IFN response of the host is beneficial or detrimental to infection is still unclear. Indeed, mice invalidated for the type I IFN receptor (IFNAR1$^{-/-}$) are more resistant to *Listeria* infection when inoculated via intravenous route, but more sensitive to an oral challenge [81,82]. In line with this, recent studies suggest that the outcome of the IFN response also depends on its timing: a delayed onset of type I IFN induction proves detrimental to the host, while IFNβ treatment in the early phase of infection shows anti-bacterial properties [83]. This suggests that type I IFN antibacterial effects can be efficient against *Listeria*, while its properties to down-regulate host defences have opposite effects at later time points [84,85].

The analysis of host gene expression reprogramming in response to infection has recently revealed that *Listeria* also induces type-III IFN (IFN-λ) in infected intestinal epithelial cells, an event so far only known for viral infections [86]. Detailed analysis has shown that production of IFN-λ requires bacterial entry into host cells, and increases with bacterial replication [87]. These findings were extended to other gram-positive bacterial species during the infection of epithelial cells and tissues [87]. So far, it is still unknown whether IFN-λ plays a positive or a negative role on the outcome of infection.

Strategies to escape or counteract innate immune responses.

L. monocytogenes has evolved ingenious mechanisms to evade the innate immune defences of its host. One such mechanism is the *O*-acetylation of muramic residues contained in the peptidoglycan, performed by the *O*-acetyltransferase OatA [88]. In a way reminiscent of the previously characterised peptidoglycan *N*-deacetylase PgdA mode of action [89], OatA increases the bacterial cell wall resistance to antimicrobial compounds such as lysozyme, thereby favouring bacterial persistence in macrophages and virulence *in vivo*. In addition, the stronger resistance of the bacterial cell wall limits the triggering of host pro-inflammatory response.

Listeria also dampens its host pro-inflammatory response through the secretion of InlC, a virulence factor of the internalin family [90] (Fig. 4A). In the cytosol, InlC directly interacts with the IKKα kinase and prevents the phosphorylation of IκB, the regulatory subunit in the NF-κB complex. As a consequence, IκB is no longer degraded in response to pro-inflammatory signals

such as TNF-α, blocking the translocation of activated NF-κB to the nucleus, as well as the downstream production of cytokines and the chemo-attraction of neutrophils to the infection site.

Epigenetics and infection: Chromatin-based regulations of host transcription

In eukaryotic cells, DNA is condensed into chromatin, which can be either in an open, transcriptionally active, or in a closed, transcriptionally silent state. An important component in the control of the host transcriptional response to infection is the open or closed status of chromatin. Recent research aiming at exploring this level of regulation in the context of *Listeria* infection broadly contributed to the emerging field of bacterial infection and epigenetics [91].

It had previously been shown that LLO as well as other bacterial pore forming toxins of the same family trigger the dephosphorylation of histone H3 (H3deP) and deacetylation of histone H4 (H4deAc) [92], which correlate with the repression of a subset of host genes (Fig. 4B). Further mechanistic investigations have highlighted that H3deP is induced by potassium efflux when the host cell plasma membrane is permeabilized [93]. In an independent pathway, this K^+ efflux also activates caspase-1 and thereby the inflammasome.

Infection by *L. monocytogenes* additionally stimulates the deacetylation of histone H3 on lysine 18 (H3K18) [70] (Fig. 4C). H3K18 is deacetylated by the NAD-dependent deacetylase sirtuin 2 (SIRT2), which upon binding of InlB to the Met receptor translocates to the nucleus and associates with the promoter of a number of genes. SIRT2 activity has a major influence on the down-regulation host genes in infected cells, including a significant number of immune-related genes. Impaired SIRT2 function is detrimental to infection in cultured cells or in a murine listeriosis model, suggesting that the newly described role of SIRT2 in gene expression reprogramming is actively exploited by the pathogen to its own benefit.

Control of the host chromatin by *L. monocytogenes* was also illustrated in another report. LntA is a recently discovered virulence factor from *Listeria monocytogenes*, belonging to the emerging family of nucleomodulins [94]. After secretion in the host cell cytosol, LntA enters the infected cell nucleus, interacts directly with a chromatin component, BAHD1, and inhibits its activity [86,95] (Fig. 4D). In parallel to the study of the bacterial effector LntA, the function of BAHD1 in non-infected cells has been explored, reinforcing the notion that bacterial pathogens may help unravelling unexpected aspects of cell physiology; BAHD1 participates in the nucleation and spreading of a novel repressive chromatin complex, which induces gene silencing when recruited at promoters [96]. During *Listeria* infection, the BAHD1-associated chromatin complex represses the expression of interferon-stimulated genes (ISG) by a so-far unknown mechanism [95]. Upon interaction with LntA, BAHD1 dissociates from the promoters of ISGs, resulting in a reactivation of these genes, and affecting the outcome of *in vivo* infections in mice. Strikingly, the expression of *lntA* is tightly regulated, thereby avoiding uncontrolled activation of host innate immune responses. Altogether, the LntA-BAHD1 interplay fine-tunes the expression of ISGs during the progression of infection.

The three latter examples significantly participated in the rise of a new field of research named patho-epigenetics, a promising area for future investigations. One of the discussed issues is whether the targeting of chromatin by *Listeria* and other pathogenic bacteria could leave a permanent imprint, so that host cells – including, perhaps, non-immune stem cells – might keep a memory of past infections. Ongoing studies should soon provide answers to this hypothesis.

Conclusions

A true Renaissance is occurring in Microbiology [97]. By using new and cutting edge

approaches such as transcriptomics, in vivo imaging, single cell analysis etc., this "New Microbiology" is generating novel concepts, including the discovery of several unsuspected RNA-based mechanisms to regulate gene expression. It is also highlighting the importance of the microbial social life and that of cell communication in microbial assemblies. The notions of persistence and dormancy are increasingly influencing the field of infection biology, which itself has been revolutionized since nearly three decades by live cell imaging. In this "New Microbiology", *Listeria* has been one of the champions and we anticipate that it will continue to be.

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Figures

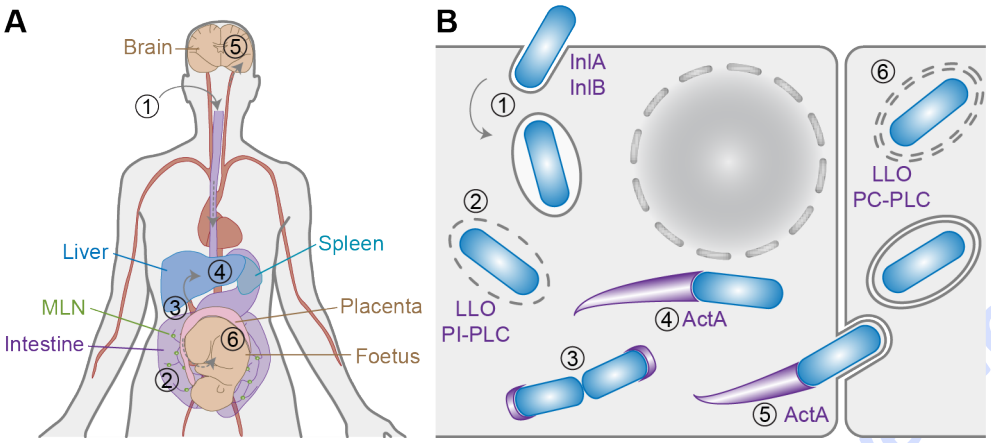

Fig. 1. Infection by Listeria monocytogenes.

A. The *in vivo* infection process. Following ingestion of contaminated food (1), bacteria colonize the digestive track. They can cross the intestinal barrier (2) and, after reaching the mesenteric lymph nodes (MLN), gain access to the systemic circulation (3). The primary target organs of the infection are the liver and spleen (4), which appear to constitute reservoirs of bacterial persistence if the infection is not controlled by immune defences. Release of bacteria into the blood stream can give rise to septicaemia. In some cases, *L. monocytogenes* cross the blood-brain barrier and reach the brain (5), resulting in meningitis or encephalitis. In pregnant women, crossing of the placental barrier (6) can lead to abortion, or generalized neonatal infection. **B. Intracellular life cycle of *Listeria monocytogenes*.** (1) *Listeria* enters into host cells via a zipper mechanism, which requires the interaction of surface internalins InlA and InlB with their respective cell surface receptors E-cadherin and Met. (2) The endocytic vacuole is ruptured via the action of secreted effectors, the pore-forming toxin listeriolysin O (LLO) and phosphatidylinositide phospholipase C (PI-PLC). (3) Bacteria can replicate in the cytosol, using cytosolic resources to their own benefit. The bacterial surface protein ActA stimulates the polymerisation of cellular actin via the recruitment of the Arp2/3 complex. This gives rise to actin comet tails, which allow intracellular motility (4) and cell-to-cell spread (5) of the bacteria. (6) Rupture of the two-membrane vacuole is mainly mediated by the action of LLO and phosphatidylcholine-specific phospholipase C (PC-PLC).

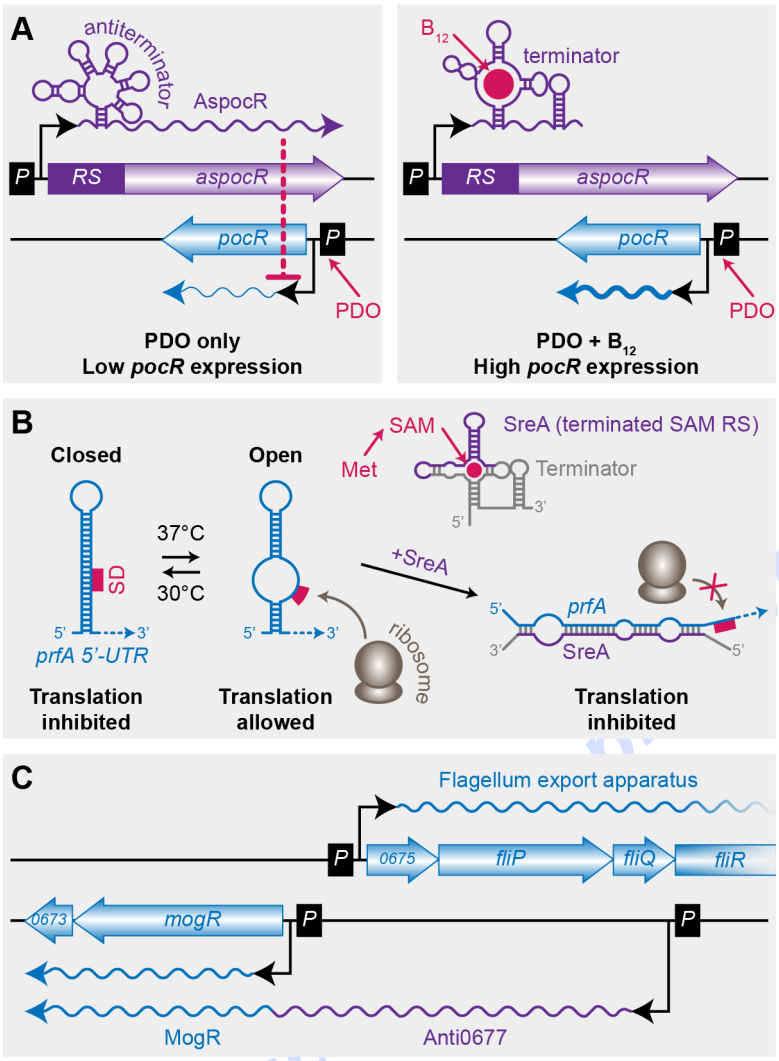

Fig. 2. Novel regulatory mechanisms in prokaryotic gene expression.

A. Model for the B_{12}-dependent regulation of *pocR* via AspocR. (*Left*) In absence of B_{12}, AspocR is transcribed and inhibits *pocR* expression by base-pairing with PocR mRNA. (*Right*) In presence of B_{12}, binding of this ligand to the riboswitch (RS) triggers the premature ranscription termination of AspocR; as a consequence, PocR mRNA can be produced. Note that transcription of *pocR* is also dependent on propanediol (PDO). **B. Regulation of prfA by *cis*- and *trans*- acting RNA elements.** The 5'-UTR of PrfA mRNA forms a closed stem loop structure at low temperatures, masking the Shine-Dalgarno (SD) sequence and thereby inhibiting ribosome binding. At 37 °C, the stem loop structure melts, desequestering the SD sequence and allowing ribosomes to initiate translation. In presence of high concentration of methionine, a S-adenosyl-methionine (SAM)-dependent riboswitch terminates transcription upstream of *lmo2419*, thus generating the 227-nucleotide sRNA SreA. By binding to the 5′ UTR of PrfA mRNA, terminated or un-terminated transcripts containing the SreA sequence block the translation of PrfA, independently of SAM binding. **C. Typical example of an excludon: the *mogR-fli* locus.** An excludon consists of divergent transcriptional units overlapped by a long antisense RNA. Anti0677 can act as a negative regulator for the *fli* operon, but its distal part also harbours information as a mRNA, contributing to the expression of *mogR*. MogR is a transcriptional repressor of flagellum genes, while Fli proteins participate in the flagellum export apparatus; the two divergent arrays of genes thus have opposite functions.

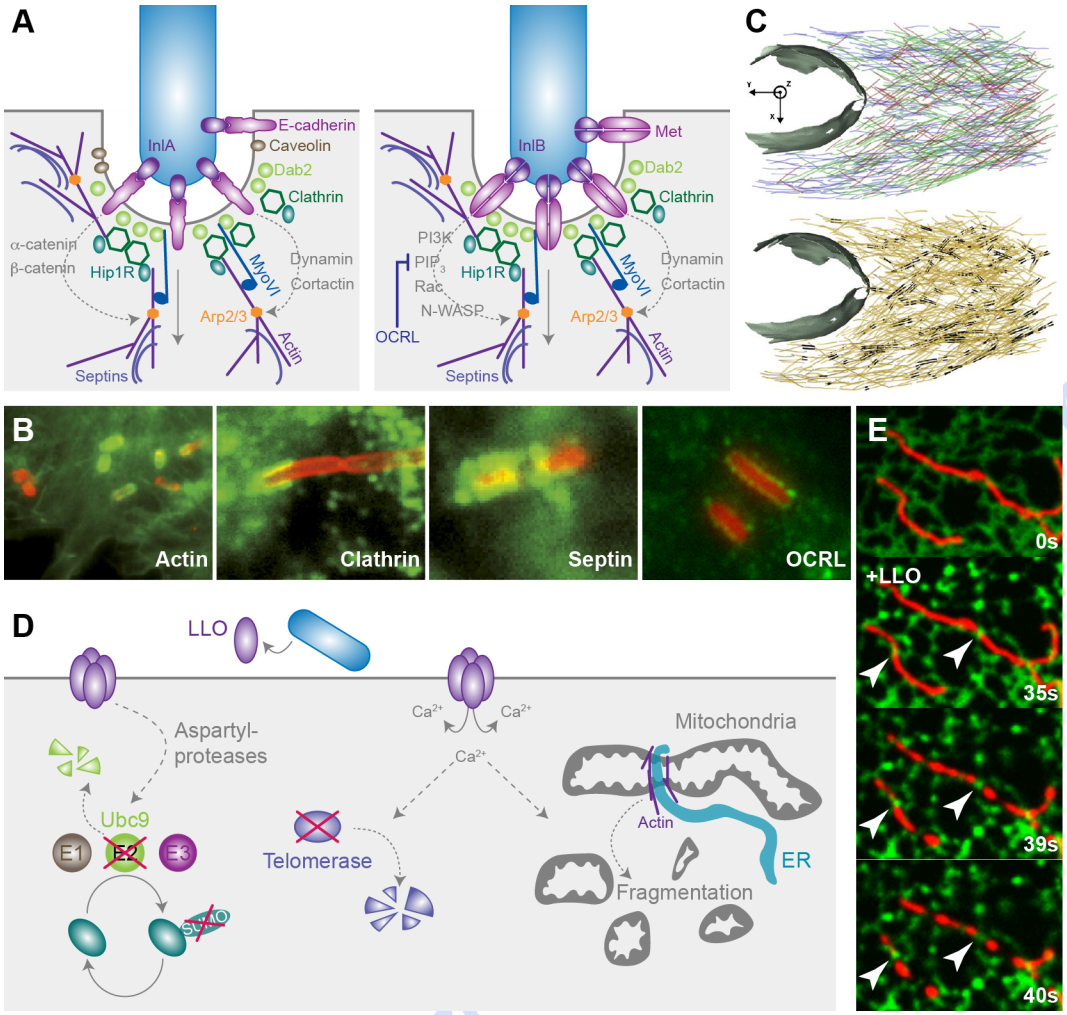

Fig. 3. Subversion of host cell functions by Listeria.

A. Proposed model for the recruitment of the endocytic machinery during *Listeria* entry. (*Left*) InlA-mediated entry. (*Right*) InlB-mediated entry. **B. Localisation of various cell components at the site of *Listeria* entry**. In each one of these independent immunofluorescence snapshots, host cell components are labelled in green; bacteria are in red, Actin, Clathrin, Septin, reprinted from [9] with permission of PNAS; OCRL, courtesy of J. Pizarro-Cerdá. **C. Three-dimensional architecture of actin filaments in *L. monocytogenes* comet tails.** Filaments were projected into the XY plane. The cell wall of the bacterium is shown in grey. (*Top*) Colours of the filaments correspond to their angle with respect to the Y-axis: 0–15° (blue), 15–30° (green), 30–45° (red). (*Bottom*) Pairs of parallel filaments are highlighted in black. Reprinted from [60] with permission of PNAS. **D. Listeriolysin O (LLO) affects protein levels and mitochondrial dynamics.** Secretion of LLO induces the degradation of the E2-SUMO ligase Ubc9, and a global de-SUMOylation of host cell proteins. Via its pore-forming activity, LLO also leads to calcium influx, which promotes the proteasome-independent degradation of telomerase, and the fragmentation of the mitochondrial network. The endoplasmic reticulum (ER) and actin participate in the Drp1-independend mechanism of mitochondrial fission. **E. The endoplasmic reticulum marks sites of mitochondrial fission.** Arrowheads indicate mitochondrial fragmentation sites marked by ER-mitochondria contact sites. Mitochondria (DsRed2-mito; red) and ER (Sec61β-GFP; green) are shown, by confocal microscopy of Sec61β-GFP cells after addition of 2 nM LLO (Courtesy of F. Stavru).

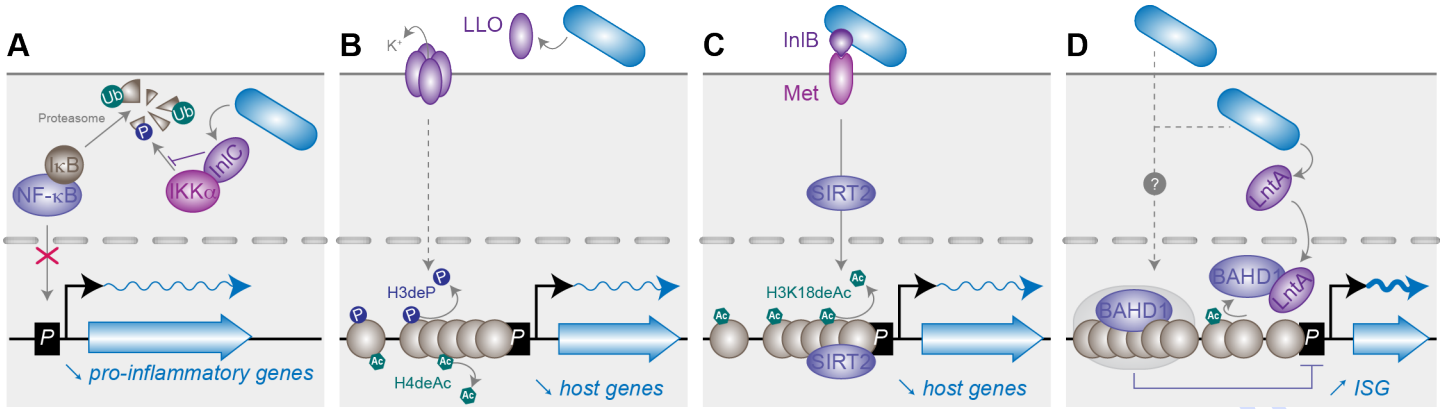

Fig. 4. Control of host gene transcription by Listeria.

A. InlC inhibits NF-κB dependent pro-inflammatory signalling. Secreted InlC interacts with the kinase IKKα, and prevents it to phosphorylate IκB. This impairs the ubiquitin (Ub)-proteasome dependent degradation of IκB, which sequesters NF-κB (p50-RelA) in the cytoplasm and dampens the induction of pro-inflammatory responses. **B. Listeriolysin O (LLO) induces histone modifications.** The pore-forming activity of LLO is responsible for an efflux of cellular potassium (K^+). This triggers a global dephosphorylation of histone 3 on serine 10 (H3deP) and deacetylation of H4 (H4deAc), and modulates the expression of host genes. **C. InlB-Met signalling targets SIRT2 to the chromatin.** Activation of Met-dependent signalling cascades by InlB triggers the translocation of the protein deacetylase SIRT2 to the chromatin, where it deacetylates histone 4 on lysine 18 (H3K18deAc). This leads to the repression of various host genes including transcriptional regulators and genes involved in innate immune responses, and promotes infection. **D. The LntA-BAHD1 interplay fine-tunes the expression of interferon-stimulated genes (ISG).** The chromatin-associated repressor BAHD1 controls the expression of ISGs during *Listeria* infection. When it is produced, LntA is targeted to the nucleus and binds directly with BAHD1. This interaction releases BAHD1 from the promoter of ISGs and allows their induction.